We may draw some quantitative conclusions from these data with respect to the effects of neutral salts on the stability of biological macromolecules. Taking the average value of $\Delta G_{tr,Am}$ from the last column of Table IV, we may estimate that the free energy of transfer of a peptide group from the interior of protein to a 1 M mono-monovalent salt solution is ~200 cal/mol more favorable than the transfer of the same group to water. The transfer of a methylene group to 1 M NaCl is ~100 cal/mol more unfavorable than transfer of this moiety to water. Since NaCl is "inert" as a conformational perturbant (*i.e.*, it neither stabilizes nor destabilizes the native conformation of biological macromolecules), we may estimate that the average "interior" residue in a protein may be represented by a peptide group and approximately two methylene units. This is in good accord with our previous conclusion (von Hippel *et al.*, 1973) that the acrylamide moiety may be used to represent the average interior group of a protein. On this basis transfer to 1 M $NaClO_4$ destabilizes the folded conformation of a protein by ~80 cal/mol of amino acid residues exposed in the transition and, to the extent that K_s is independent of salt concentration, by, *e.g.*, ~0.5 kcal/mol of residues exposed to 6 M $NaClO_4$. In future studies we plan to test these predictions for macromolecules for which the details of thermally induced unfolding transitions are known or can be surmised.

References

Hamabata, A., Chang, S., and von Hippel, P. H. (1973), *Biochemistry 12*, 1278.

Hamabata, A., and von Hippel, P. H. (1973), *Biochemistry 12*, 1264.

Klotz, I. M., and Franzen, J. S. (1962), *J. Amer. Chem. Soc. 84*, 3461.

Long, F. A., and McDevit, W. F. (1952), *Chem. Rev. 51*, 119.

Mukerjee, P. (1967), *Advan. Colloid Interface Sci. 1*, 241.

Nandi, P. K., and Robinson, D. R. (1972a), *J. Amer. Chem. Soc. 94*, 1299.

Nandi, P. K., and Robinson, D. R. (1972b), *J. Amer. Chem. Soc. 94*, 1308.

Robinson, D. R., and Jencks, W. P. (1965), *J. Amer. Chem. Soc. 87*, 2470.

Schrier, E. E., and Schrier, E. G. (1967), *J. Phys. Chem. 71*, 1851.

von Hippel, P. H., Peticolas, V., Schack, L., and Karlson, L. (1973), *Biochemistry 12*, 1256.

von Hippel, P. H., and Schleich T. (1969a), *in* Biological Macromolecules, Vol. II, Timasheff, S., and Fasman, G., Ed., New York, N. Y., Marcel Dekker, p 417.

von Hippel, P. H., and Schleich, T. (1969b), *Accounts Chem. Res. 2*, 257.

von Hippel, P. H., and Wong, K.-Y. (1964), *Science 145*, 577.

Model Studies on the Effects of Neutral Salts on the Conformational Stability of Biological Macromolecules. IV. Properties of Fatty Acid Amide Micelles†

Alberto Hamabata,‡ Susan Chang, and Peter H. von Hippel*

ABSTRACT: In this article, the anomalous alterations in the rate of change of the solubility of *n*-hexanamide with temperature in water and aqueous salt solutions which were observed previously (Hamabata, A., Chang, S., and von Hippel, P. H., (1973), *Biochemistry 12, 1271*) are shown to be due to the onset of micelle formation in this system. Relative changes in the fluorescent intensity of the dye 8-anilino-1-naphthalenesulfonic acid (ANS), which shows greatly increased fluorescent quantum yields on transfer from an aqueous to a nonpolar environment, are used to monitor micelle formation and to establish critical micelle concentration (cmc) values. The fact that micellar aggregates of fairly discrete size are being established at the cmc is confirmed by preliminary sedimentation velocity experiments. Cmc values are measured fluorimetrically for hexanamide micelles as a function of the concentration of various neutral salts, and it is known that all obey the empirical relation: $\log (\text{cmc}) = \log (\text{cmc})_0 - k_{s,\text{cmc}} C_s$. The parameter k_s varies with salt type, decreasing in approximately the usual Hofmeister order: $NaCl \simeq NaBr > NaI > NaClO_4$. It is shown that $k_{s,\text{cmc}}$ is equivalent to the salting-out coefficients (K_s) obtained from solubility data in preceding articles, and free energies of transfer from water to 1 M salt solutions are calculated from the $k_{s,\text{cmc}}$ data. These values correspond to the net effect of the transfer of that portion of the hexanamide monomer which changes microenvironments in going from the free monomer to the micellar state, and the data obtained are compared with free energies of transfer measured on the same groups in macroscopic systems. The results are used to interpret micelle structure and to illustrate some of the ambiguities which accompany the notions of "interior," "exterior," and "surface" on the macromolecular size scale.

In the preceding article (Hamabata *et al.*, 1973) we reported studies on the effects of various neutral salts on the solubility of fatty acid amides of varying chain length in aqueous solutions. We observed that the solubilities of these amides

† From the Institute of Molecular Biology and the Department of Chemistry, University of Oregon, Eugene, Oregon 97403. *Received September 27, 1972.* This research was supported by U. S. Public Health Service Research Grant No. GM-15792 (to P. H. v. H.) and U. S. Public Health Service Fogarty International Postdoctoral Research Fellowship No. FO5TW1603 (to A. H.).

‡ Present address: Department of Biochemistry, Instituto Politecnico Nacional, Mexico City, Mexico.

behaved predictably under most conditions, but that in certain salts at elevated temperatures plots of the dependence of solubility on temperature, salt concentration, and other independent variables demonstrated abrupt discontinuities, suggesting a concentration-dependent change in the molecular properties of the system. In this paper we demonstrate that these points of abrupt change in the solubility of *n*-hexanamide are due to the onset of micelle formation, and mark the cmc (critical micelle concentration) for the system under these conditions.

Fatty acid amide micelles are particularly good models for the questions to which we are addressing ourselves in this series of articles, since, as will be shown, they are microphases of about the size of biological macromolecules, with a discrete "inside" containing primarily the aliphatic "tail" of the amide monomers and an "outside" consisting mostly of the polar amide "head" groups. By studying the effect of neutral salts on the cmc values of such systems, we can therefore examine directly the effects of these salts on the free energy of transfer of the hydrocarbon tails of the amide monomers from the nonpolar micelle "interior" into the aqueous surround, in systems for which the concepts of micellar "interior," "exterior," and "surface" are subject to many of the same ambiguities which apply to biological macromolecules. The results are compared directly with the free energy of transfer results obtained by macroscopic chromatographic and solubility measurements in the previous articles of this series.

Materials and Methods

Chemicals. All salts used in this study were reagent grade and were used without further purification, except that solutions were filtered by passage through 0.45-μ Millipore filters. *n*-Hexanamide was obtained from Eastman (lot 711A) and recrystallized from cyclohexane prior to use. 8-Anilino-1-naphthalenesulfonic acid (ANS) was purchased from Aldrich. Sodium cacodylate (0.01 M) (Matheson, Coleman and Bell) was used as a buffer at pH 7.0 in all salt solutions. NaI solutions contained 0.005 M $Na_2S_2O_3$ to prevent oxidation of iodide.

Fluorescence Measurements. Relative fluorescent intensities were obtained with a recording spectrofluorimeter (Hitachi MPF-2A spectrometer equipped with a Hitachi recorder and a Perkin-Elmer 150 Xenon power supply). The fluorimeter was operated at an effective excitation slit width of 5 nm (0.65 mm) and an emission slit width of 20 nm (2.68 mm). The excitation wavelength was 365 nm, and the emission slit was followed by a no. 43 filter to cut off stray light below 430 nm. The cell compartment of the fluorimeter was thermostated at 50° (±0.5°) for all measurements; 10 × 10 mm cells were used in most determinations, though identical results were obtained with experiments repeated in 2.9 × 2.9 mm cells.

Changes in ANS fluorescence on micelle formation were used to establish the critical micelle concentration for hexanamide at various concentrations (see Results). The same ANS concentrations (usually 10^{-4} M) in 30% ethanol (v/v) were used as standards to define 100% fluorescence in the relative fluorescence measurements. The uncorrected ANS spectrum in water shows an emission maximum at 520 nm, which shifts to 510 nm in 30% ethanol.

Results

Experiments discussed in the preceding article had shown that the solubility of *n*-hexanamide increases with temperature, and that in a particular salt solution the apparent *rate* of this solubility change increases abruptly at a critical temperature (*e.g.*, see Figure 1 and Figure 8 of Hamabata *et al.*, 1973). This suggested that the solution had undergone some form of phase transition. Since hexanamide is a relatively amphipathic molecule (characterized by a relatively polar "head" group and a nonpolar "tail"), by analogy with the properties of detergent molecules and other micelle-forming species it seemed reasonable to speculate that micelle formation had taken place, though perusal of a rather complete compendium (Mukerjee and Mysels, 1971) of known micelle-forming systems lists no amides.

The existence of micelles is generally proven by demonstrating a critical micelle concentration (cmc) at which there is an abrupt change in solution properties with increasing monomer concentration. These solution property changes generally correspond to the appearance of an aggregated (and often quite monodisperse) species in equilibrium with free monomer. The reaction is usually formulated

$$n(\text{monomer}) \rightleftharpoons (\text{monomer})_n \qquad (1)$$

for micelles containing *n* monomer units per micellar aggregate. Obviously, according to this formulation, micelle formation is highly cooperative (cooperativity increasing with *n*), which accounts for the abrupt discontinuities in physical properties of micelle-forming solutions observed at the cmc. However, many workers have debated the question of whether in some micelle-forming systems extensive pre-cmc association reactions (formation of stable dimers, trimers, etc.) might not provide a number of fairly populated intermediates in the micelle-forming process. This question has recently been reviewed in detail by Mukerjee (1967). Since the driving force for micelle formation is the creation of a microphase with an essentially water-free interior, into which the nonpolar tails of the monomers may be transferred with a consequent favorable free-energy change for the system as a whole (partially offset by the unfavorable entropy of mixing associated with this process), the micellar aggregates obviously need to be large enough to permit the formation of such a nonpolar "interior" volume. The concensus for most systems which have been studied with care seems to be that eq 1 is a reasonable representation of the micelle-forming process, and that stable pre-cmc association complexes, if present, occur at negligible concentrations (Mukerjee, 1967). Data available for this system (*e.g.*, Figure 8 of Hamabata *et al.*, 1973, and Figure 1) are consistent with this conclusion.

Critical micelle concentrations can be defined in a number of ways. Physical techniques that have been used to detect the onset of micelle formation include sedimentation, light scattering, osmotic pressure, conductivity, and surface tension measurements (see Mukerjee, 1967, for further details). A fluorescent dye that changes fluorescent intensity with changes in solvent polarity can serve as a particularly useful indicator of micelle formation (Mukerjee and Mysels, 1971). ANS is one of a number of polycyclic aromatic compounds that are virtually nonfluorescent in aqueous solution, and become highly fluorescent in nonpolar solvents. Thus, the quantum yield of ANS in water is 0.004, and rises to 0.22 in methanol, 0.37 in ethanol, and 0.46 in *n*-propyl alcohol. The corrected emission maximum also shifts toward the blue as the polarity of the solvent is decreased: λ_{max} 515 nm in water, 484 nm in methanol, 476 nm in ethanol, and 468 nm in *n*-propyl alcohol (Stryer, 1965). Based on these properties, ANS has been ex-

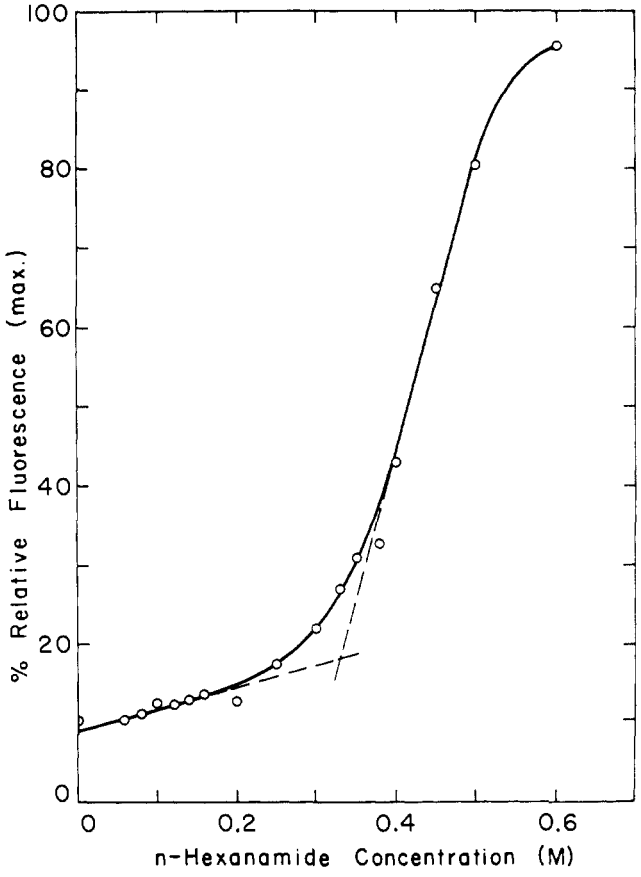


FIGURE 1: Relative maximum fluorescence of ANS in 1 M $NaClO_4$ at 50° as a function of *n*-hexanamide concentration. Cmc is defined as the intercept of the dashed straight lines (see text).

tensively used as a probe of hydrophobic sites in proteins (*e.g.*, Stryer, 1965) and in Figure 1 we show that it can be used to measure cmc values for micelle-forming systems.

In Figure 1 the maximum relative fluorescence intensity at 50°, for a series of solutions containing 10^{-4} M ANS (and in this case 1 M $NaClO_4$), plus varying concentrations of hexanamide, is plotted against hexanamide concentration. We may see that there is essentially no change in fluorescence with hexanamide concentration at low monomer concentrations, and that the maximum relative fluorescent intensity increases, abruptly and cooperatively, to 10–20 times the initial value as the cmc is passed (with an associated shift of the uncorrected λ_{max} from 520 to ~500 nm). As shown in Figure 1, we define the cmc of the system as the intercept of the two straight lines passed through the pre- and the post-cmc data, respectively. Cmc values were defined by the same technique for the various neutral salt systems studied (see below). Control experiments were carried out to show that the measured cmc values are independent of ANS concentrations at the levels used (*i.e.*, to show that the presence of this amount of ANS does not measurably change the stability of the micelles).

The formation of micellar aggregates of discrete size was demonstrated independently by showing the development of a sharp sedimentation velocity peak in the analytical ultracentrifuge, with an initial $s_{20,w}$ of ~5 S, as the concentration of hexanamide was increased past the cmc. No peak was seen at sub-cmc hexanamide concentrations. Reliable estimates of micelle size could not be made from these data, since the sedimentation coefficient varies appreciably with position in the ultracentrifuge cell, suggesting that the micelle size is probably quite pressure dependent.[1] The apparent density of hexanamide solutions also shows a discontinuity at the cmc. These observations will be pursued in a future study.

TABLE I: Critical Micelle Concentrations for *n*-Hexanamide in Aqueous Salt Solutions at 50°.[a]

Salt Type	Salt Concn (M)					
	0[b]	0.5	1.0	2.0	3.0	4.0
$NaClO_3$	0.406		0.328	0.240	0.200	0.161
NaCl	0.406	0.317	0.247			
NaBr	0.406	0.318	0.266			
NaI	0.406	0.350	0.310			
$NaClO_4$ + NaCl[c]	0.406		0.284	0.214		
$NaClO_4$ + 2 M formamide	0.492			0.306	0.259	0.195
$NaClO_4$ + 4 M formamide						0.258

[a] Cmc values are in units of moles/liter. Standard deviations where multiple measurements were made are ~±5%; cmc, critical micelle concentration. [b] Cmc in 0.01 M sodium cacodylate buffer, pH 7.0. [c] Measured in an equimolar mixture of $NaClO_4$ and NaCl. "1.0 M" means 0.5 M of each salt, etc.

Critical micelle concentrations for hexanamide have been measured as defined in Figure 1 for several concentrations of neutral salts. Qualitatively similar maximum relative fluorescence curves as a function of hexanamide concentration were obtained for all solutions tested, though the transitions appeared to become somewhat sharper (more cooperative) at higher salt concentrations, suggesting that somewhat larger micelles might be favored under these conditions. Cmc values derived in this fashion are summarized in Table I and Figure 2.

Several workers have shown empirically for a variety of micelle–salt systems that log (cmc) appears to show a linear dependence on salt concentration, with the slope varying with salt concentration (see Mukerjee, 1965, and more recently Ray and Némethy, 1971). Figure 2 confirms that this relationship applies to the hexanamide data for several neutral salts. Thus, we may write

$$\log (\text{cmc}) = \log (\text{cmc})_0 - k_{s,\text{cmc}} C_s \qquad (2)$$

where $(\text{cmc})_0$ represents the cmc of hexanamide under the conditions of the experiment in buffer alone, C_s is the molarity of salt of type s, and $k_{s,\text{cmc}}$ is the slope of the resultant straight line. Table I and Figure 2 show that all the salts tested decrease the cmc of the hexanamide system with increasing salt concentration, suggesting in terms of the micelle model implied in eq 1 that the free energy of transfer of the nonpolar tail of the hexanamide monomer from the nonaqueous micellar interior to the aqueous surround becomes progressively more unfavorable in the presence of added salt.

However, Figure 2 shows that $k_{s,\text{cmc}}$ varies for different

[1] Similar and much more extensive observations have been made of the pressure dependence of micelle size in ultracentrifugal studies on other micelle-forming systems by Yphantis *et al.* (1972).

TABLE II: Salt Effects on Critical Micelle Concentrations of *n*-Hexanamide at 50°.

Salt	$k_{s,cmc}$ (M^{-1})[a]	$\Delta G_{tr,cmc}$ (kcal/mol)[b]	$\Delta G_{tr,CH_2,av}$ (kcal/mol)[c]
$NaClO_4$	+0.10	+0.16	+0.06
NaI	+0.12	+0.19	
NaBr	+0.21	+0.33	
NaCl	+0.21	+0.33	+0.10
$NaClO_4$ + NaCl	+0.16	+0.25	+0.08
$NaClO_4$ + 2 M formamide	+0.10	+0.16	

[a] Defined as the slope of a plot of log (cmc) *vs.* salt concentration (see text and Figure 2). [b] Calculated by eq 4 of Hamabata *et al.*, 1973. [c] From Table IV of Hamabata *et al.*, 1973.

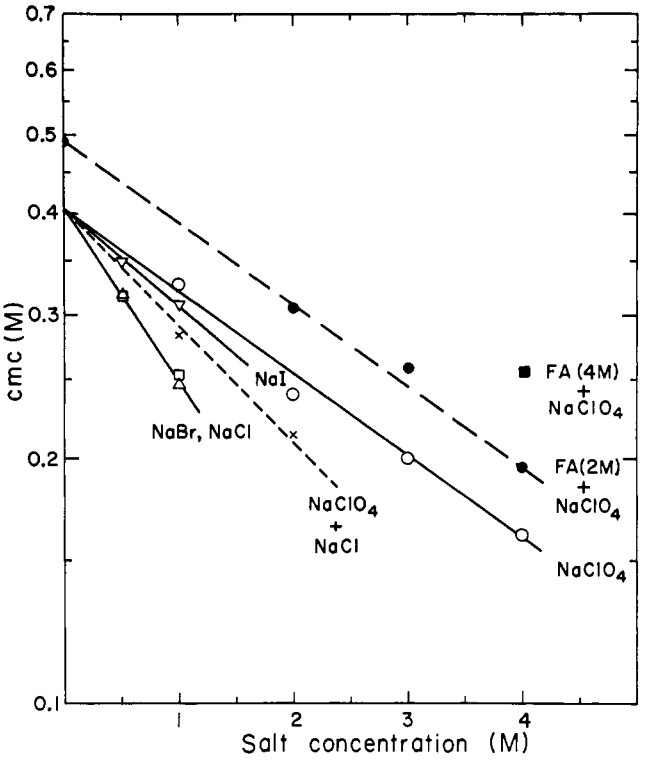


FIGURE 2: Log of the critical micelle concentration of *n*-hexanamide as a function of concentration of various neutral salts: (○) $NaClO_4$; (▽) NaI; (□) NaBr; (△) NaCl; (×) $NaClO_4$ + NaCl; (●) $NaClO_4$ + 2 M formamide (FA); (■) $NaClO_4$ + 4 M FA.

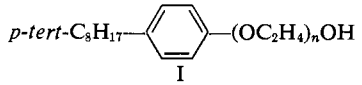
salts, increasing in the order: $k_{NaCl,cmc} \simeq k_{NaBr,cmc} > k_{NaI,cmc} > k_{NaClO_4,cmc}$. That is, $NaClO_4$ increases the free energy of transfer of the tail portion of hexanamide from the micelle interior to the aqueous environment less than comparable concentrations of NaI, etc. Values of $k_{s,cmc}$ for the various salts tested are summarized in Table II.

Figure 2 and Table II also show that equimolar mixtures of $NaClO_4$ and NaCl show $k_{s,cmc}$ values half way between those for NaCl and $NaClO_4$, confirming for this system the additivity of the observed salt effects. It is also shown that the addition of formamide to the hexanamide–$NaClO_4$ system does not change $k_{NaClO_4,cmc}$, but does increase log $(cmc)_0$. The significance of this observation will be considered below.

Discussion

In this and the preceding article, we have shown that *n*-hexanamide is capable of forming micelles of relatively discrete size, and that the stability of these micelles as reflected in values of cmc can be altered by adding neutral salts to the aqueous environment. Furthermore, the effectiveness of the various salts in altering the cmc (as reflected in $k_{s,cmc}$) varies specifically with salt type, and appears to follow the Hofmeister anion series. Data in the preceding article suggest that micelle formation also occurs with the *n*-pentanamide systems, though at appreciably higher cmc values. Increases in cmc with decreasing nonpolar chain length have been observed in many other micelle-forming systems (see Mukerjee, 1967).

Similar observations of the effects of neutral salts and denaturing agents on micellar cmc values have been made by Ray and Némethy (1971) and Gratzer and Beaven (1969). Both of these studies were conducted on a nonionic detergent (*p-tert*-octylphenoxy(polyethoxy)ethanol) of fairly complicated structure (I), containing a branched nonpolar tail group and a long polyethoxy "head" group, separated by a benzyl moiety.



I

The complex nature of this monomer makes it rather difficult to define the head and tail groups unambiguously, or to calculate free energies of transfer for constituent groups in the nonpolar tail. Earlier work (see Ray and Némethy, 1971, for detailed references) had focused on salt effects on ionic detergent micelles, which present prohibitive interpretative problems because of the strong electrostatic interactions between the charged head groups. The fatty amide system appears to be simpler in these respects.

We picture hexanamide micelles as structures in which most of the methyl and methylene groups of the hydrocarbon tail of the monomers interact to form a nonpolar "oil-drop" microphase, with the amide dipole presumably located on the surface and thus in significant contact with the aqueous environment. In this simplified view only the microenvironment of the tail groups is changed by micelle formation, except for some crowding of the amide dipoles on the micellar surface. To set up a compensatory crowding of the amide head groups in the monomer state, we have measured the cmc of hexanamide as a function of $NaClO_4$ concentration in the presence of 2 M formamide (see Figure 2). The slope of the log (cmc) *vs.* $NaClO_4$ concentration line is not changed by the addition of formamide, suggesting that formamide has little salting-in or salting-out effect on the monomer tail groups (or alternatively on the degree of exposure of the tail groups in the micelle), but probably increases the cmc by stabilizing the free monomers by amide–amide (hydrogen binding or dipole–dipole) interactions in the solution. The one 4 M formamide point in Figure 2 indicates that this effect increases further at higher formamide concentrations.

By analogy with the salting-out constants calculated in the preceding article, the fact that all the salts tested show positive $k_{s,cmc}$ values is also in qualitative accord with our assumption that most, if not all, of the groups transferred from an aqueous to a nonaqueous environment on micelle formation

are the methyls and methylenes of the nonpolar tail. In terms of this picture we may equate $k_{s,cmc}$ with the values of K_s calculated from the Setschenow equation (see Hamabata *et al.*, 1973), and calculate free energies of transfer from water to 1 M salt for that fraction of the hexanamide monomers which change their environment on micelle formation, as done previously for the amide solubility data (Hamabata *et al.*, 1973; eq 4). The results of such calculations, *for these data obtained at 50°*, are also shown in Table II.

The last column of Table II, in which we list the average values for the free energy of transfer of a methylene group from water to 1 M $NaClO_4$ or NaCl, as obtained from differential amide solubility data (Table IV; Hamabata *et al.*, 1973), suggests that our picture of the micelle structure may be oversimplified. In the preceding article it was shown that $\Delta G_{tr,CH_2}$ is approximately independent of temperature between 6 and 25°. *If* this independence of temperature of $\Delta G_{tr,CH_2}$ extends to 50°, then these data suggest that a *net* transfer of only *ca.* three methylene group equivalents per hexanamide monomer is taking place on micelle formation. Alternatively, we may speculate that micelle formation *does* involve a fractional burial of the amide group, which results in a *negative* contribution to the total $\Delta G_{tr,cmc}$ from water to 1 M salt, and thus partially offsets the larger positive ΔG_{tr} expected for the total nonpolar tail of the hexanamide monomer. A more detailed consideration of the origins of such possible cancellation effects in terms of the location of the "micellar surface" is presented by Ray and Némethy (1971) for the *p-tert*-octylphenoxy(polyethoxy)ethanol–salt systems they have examined. Further experimental work will be required to resolve these ambiguities.

References

Gratzer, W. B., and Beaven, G. H. (1969), *J. Phys. Chem. 73*, 2270.

Hamabata, A., Chang, S., and von Hippel, P. H. (1973), *Biochemistry 12*, 1271.

Mukerjee, P. (1965), *J. Phys. Chem. 69*, 4038.

Mukerjee, P. (1967), *Advan. Colloid Interface Sci. 1*, 241.

Mukerjee, P., and Mysels, K. J. (1971), *Nat. Stand. Ref. Data. Ser., Nat. Bur. Stand. No. 36.*

Ray, A., and Némethy, G. (1971), *J. Amer. Chem. Soc. 93*, 6787.

Stryer, L. (1965), *J. Mol. Biol. 13*, 482.

Yphantis, D. A., Dishon, M., Weiss, G., and Johnson, M. (1972), 16th Annual Meeting of The Biophysical Society, Toronto, Canada, Abstract SaAM-A7.

Minor Components of Cytoplasmic Ribonucleic Acid from Normal and Regenerating Rat Livers†

Suzanne Levin‡ and Nelson Fausto*

ABSTRACT: RNA from rat liver cytoplasm (mitochondria free) was analyzed by sucrose gradient centrifugation followed by electrophoresis in agarose–acrylamide gels. In addition to 28S and 18S ribosomal RNA we detected four minor RNA species of molecular weights ranging from 8×10^5 to 1×10^6. The minor RNA components are included in the "18S peak" of conventional sucrose gradients but can be separated by electrophoresis on 0.5% agarose–3.0% acrylamide gel slabs. These RNA species represent approximately 8% of the total ribosomal (18 + 28S) RNA in rat liver and appear to be a structural component of large ribosomal subunits in which pairs of the minor RNA components may replace intact 28S RNA. Minor RNA bands were detected in polysomes and "native" ribosomal subunits but not in RNA obtained from free monomers. The labeling kinetics of the minor RNA components from livers of partially hepatectomized rats and from fed and starved intact rats argues against these RNA species being precursors to 18S RNA or products of degradation of "old" 28S ribosomal RNA. The results show that the minor RNA species are labeled in parallel with and appear to decay at a slower rate than both 28S and 18S ribosomal RNAs.

Sucrose-gradient centrifugation of rat liver cytoplasmic RNA reveals the presence of three stable species of RNAs with sedimentation coefficients of 28, 18, and 4 S. More detailed analyses of liver cytoplasmic RNA by electrophoresis in polyacrylamide gels show in addition to the major RNA species one or more RNA bands located between 28 and 18S RNA (Dingman *et al.*, 1970; Aaij *et al.*, 1971; Takagi *et al.*, 1971). The biological functions, if any, of such minor RNA species can only be determined after their intracellular distribution and labeling kinetics are known. In this paper we describe a study of these minor RNA species in normal and regenerating rat liver using polyacrylamide–agarose gel electrophoresis.

Experimental Section

Animals. Male albino rats (Holtzman Co., Madison, Wis.) weighing 160–200 g were used in all experiments. The rats were maintained in a temperature-controlled room with alternating 12-hr cycles of light and dark. Partial hepatectomies were performed using the method of Higgins and

† From the Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island 02912. *Received November 6, 1972.* This work was supported by Grants NP-52B from the American Cancer Society and AM-14706 from the National Institutes of Health.

‡ Public Health Service Predoctoral Trainee (GM-00329). Present address: Stockton State College, Pomona, N. J.